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Title: The Brain's Kryptonite: Overview of Punctate White Matter Lesions in Neonates

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ABSTRACT

With increasing advances in the field of medical brain imaging, the known spectrum of white matter lesions has expanded, and we can now assess the presence of punctate white

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matter lesions (PWML). These focal small lesions are quite frequently detected in the preterm infant and in full-term infants with congenital heart malformations with, some studies reporting a link between these lesions and adverse long-term outcomes. The etiology of PWML has sparked a lot of questions over the years, some of which still remain unanswered. This narrative review will bring an overview of current knowledge and their significant clinical importance in the newborn brain.

Abbreviations: cUS, cranial Ultrasound; TEA, Term Equivalent Age; DTI, Diffusion Tensor Imaging; DWI, Diffusion Weighted Imaging; FA, Fractional Anisotropy; MRI, Magnetic Resonance Imaging; PVL, Periventricular Leukomalacia; PWML, Punctate White Matter Lesions; SWI, Susceptibility-Weighted Imaging

Keywords: punctate white matter lesions, neonate, brain imaging, outcome, MRI, DTI, preterm infants, birth asphyxia, congenital heart malformation.

1. Introduction

Over the past years, magnetic resonance imaging (MRI) has been implemented as an important tool to assess the integrity of the newborn brain in clinical situations such as birth asphyxia, but also to study brain growth and vulnerability of preterm infants and term infants with congenital heart disease. Along with cranial ultrasound (cUS), which remains part of the standard clinical assessment, the goal of these imaging techniques is to screen for brain lesions in critically-ill infants and in some cases to determine their prognosis and outcome. cUS is an efficient method to diagnose severe abnormalities such

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as cystic periventricular leukomalacia (PVL) or brain hemorrhage (Rutherford et al., 2010). However, with the increased use of antenatal steroids therapy and the refinement of ventilatory support in preterm infants (Hamrick et al., 2004; Rutherford et al., 2010), a decline of severe hemorrhagic lesions and a reduction in the severity and incidence of cystic PVL has been observed. Indeed, cystic PVL is currently reported in about 3% of very low birth weight infants (Van Haastert et al., 2011), whereas non-cystic white matter injuries are more common and occur in up to 79% of very preterm infants (Maalouf et al., 1999; Counsell et al., 2003; Miller et al., 2003; Volpe et al., 2011; Van De Looij et al., 2012). In the preterm brain, neuroimaging studies have shown that cUS has low sensitivity to predict motor and cognitive impairments in the absence of extensive visible damage (Ancel et al., 2006; Hintz et al., 2015; Tusor et al., 2017), and is less sensitive than MRI to detect small non-cystic white matter lesions (Kato et al., 2012; Benders et al., 2014) (see Figure 2). These areas of focal non-cystic white matter defects were first considered as a milder form of non-cystic PVL. They were also thought to share the same etiology as PVL (Volpe, 2001; Back et al., 2005; Segovia et al., 2008; Rutherford et al., 2010), although that remains to be validated.

This review aims to focus on punctate white matter lesions (PWML), a specific form of focal non-cystic white matter injury, and to provide an overview of the current knowledge on PWML in the neonatal period. Literature search was first performed on the PubMed database for all articles published before 2018-06-30 containing the specific keyword or expression "punctate white matter lesion". We focused on lesions in both preterm and at term neonates and their outcomes. Once all

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primary articles were consulted, their references were subsequently exhaustively screened for secondary references related to PWML.

2. PWML definition and evolution

PWML are focal non-cystic lesions detected in the unmyelinated white matter, which can be hemorrhagic or non-hemorrhagic. PWML are frequent brain abnormalities occurring in 18-35% of preterm infants (Inder et al., 2003; Miller et al., 2005; Dyet et al., 2006; Ramenghi et al., 2007; Kersbergen et al., 2014; Wagenaar et al., 2017; Qi et al., 2018). Although PWML are not often identified in term newborns, some medical conditions have been associated with these lesions. This is the case of congenital heart disease (Guo et al., 2019), detectable before cardiac surgery (Childs et al., 2001; Li et al., 2008; Swarte et al., 2009; Block et al., 2010; Ortinau et al., 2012), or of newborns with hypoxic ischemic injury with an incidence of 5.7% (Niwa et al., 2011). However, it is possible that PWML are far more frequent. For instance, newborns exposed in-utero to antidepressants, who are not normally scanned were found to have white matter lesions (De Vries et al., 2013).

PWML can develop as a standalone brain abnormality but is usually coupled with other types of brain insults, such as diffuse or cystic white matter lesions and hemorrhagic injuries (Bassi et al., 2011; Niwa et al., 2011; Kersbergen et al., 2014; Tusor et al., 2017). Neonates presenting with both PWML and intraventricular hemorrhage were often among the sickest (Wagenaar et al., 2017). Intraventricular hemorrhage and PWML also share similar clinical risk-factors such as respiratory distress syndrome, with a need for respiratory support (Wagenaar et al., 2017). Indeed, preterm newborns with PWML are more likely to have been intubated at birth, to have received surfactant and to

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be mechanically ventilated for a longer period of time than neonates without PWML (Wagenaar et al., 2017).

Perinatal clinical factors for PWML include: prenatal infection-inflammation and hypoxic-ischemic processes (Volpe, 2001; Hagberg et al., 2002; Back et al., 2005; Benders et al., 2014). Maternal urinary tract infection during pregnancy was linked to the presence of PWML (Tusor et al., 2017). Two studies also reported a link between the presence of PWML and a greater birth weight (non-corrected for GA) (Tusor et al., 2017; Wagenaar et al., 2017). Wagenaar et al. (2017) suggested that this surprising result may be explained by the fact that children born later, with a greater birth weight, were scanned earlier in life, when PWML were more prone to be detected on MRI, which may also be the case in the study by Tusor et al. (2017). Interestingly, postnatal sepsis was not found to be specifically associated with the occurrence of PWLM in the preterm (Rutherford et al., 2010; Bassi et al., 2011; Benders et al., 2014).

Using MRI, PWML are usually identified as small focal non-cystic spots of increased signal intensity on T1-weighted images with or without decreased signal intensity on T2-weighted images (Cornette et al., 2002; Niwa et al., 2011) (see Figure 1). PWML are most commonly found in the central white matter regions including the corona radiata, optic radiations and corticospinal tracts, followed by periventricular posterior regions (Cornette et al., 2002; Rutherford et al., 2010; Guo et al., 2017; Tusor et al., 2017). By contrast, they are less common in the region anterior to the frontal horn of the lateral ventricles (Cornette et al., 2002; Guo et al., 2017, 2019).

Insert Figure 1

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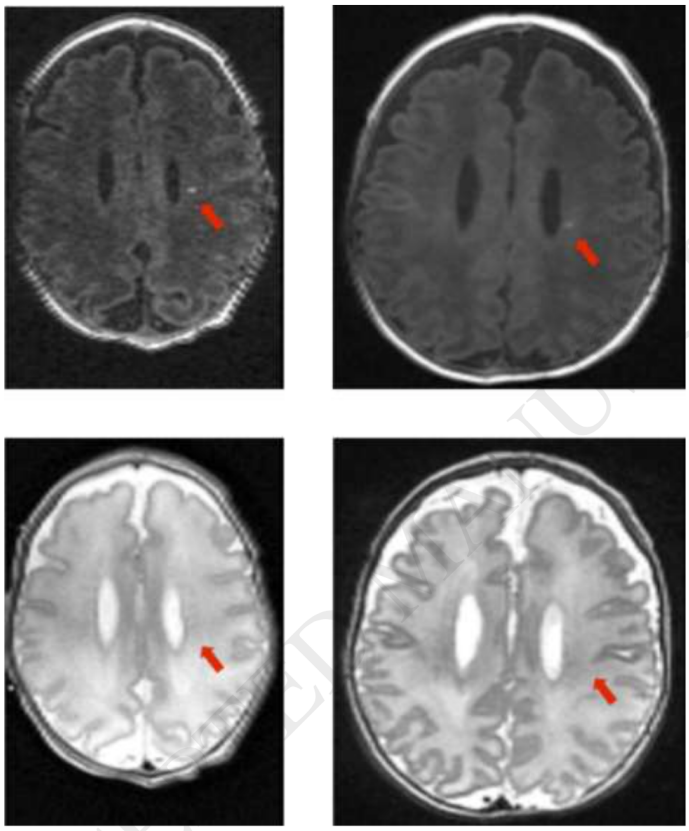

Figure 1: Punctate white matter lesions (red arrows) as seen in axial T1-weighted (top row) and T2-weighted (bottom row) of the same preterm infant born at 29 weeks of gestation imaged for the first time at 33 weeks (left column), and reimaged at 37 weeks of gestation. Acquisition resolution at 1.2mm x 0.78mm x 0.78mm. Note how PWML lesions are readily

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identifiable on T1-weighted images and can be hard to detect on T2-weighted images

In preterm infants, PWML are detected in greater numbers during the first weeks of life (see Figure 1) (Miller et al., 2005; Kersbergen et al., 2014; Martinez-Biarge et al., 2016; Guo et al., 2017; Wagenaar et al., 2017; Qi et al., 2018). At term-equivalent age (TEA), PWML become smaller, less frequent, and ultimately more difficult to detect or even disappear especially when engulfed by ventricular dilatation secondary to atrophy (Cornette et al., 2002; Dyet et al., 2006; Rutherford et al., 2010; Niwa et al., 2011; Kato et al., 2012; Lodygensky et al., 2012; Kersbergen et al., 2014; Wagenaar et al., 2017). Indeed, in 26 extremely preterm infants with PWML, only 33% of the PWML persisted at TEA (Dyet et al., 2006). It is postulated that PWML identified during the first weeks of life result in early glial scarring or necrosis with mineralization at TEA (Wagenaar et al., 2017). Indeed, in full-term infants suffering from neonatal seizures set off by bi-allelic *SLC13A5* mutations, PWML disappeared at the age of 6 months and were replaced by gliotic scarring, visible on MRI scans at the age of 18 months (Weeke et al., 2017).

3. Classifications of white matter lesions

There are multiple scoring systems on the severity of white matter lesions in preterm infants in the literature (Table 1). Childs et al. (2001) and Cornette et al. (2002) were the first to establish a scoring system that would classify focal non-cystic periventricular white matter injuries. Cornette's classification is based on: number; size; appearance (organized into clusters, linear or mixed-type pattern); location (anterior to the frontal horn of the lateral ventricles (anterior region), posterior to the occipital horn of the lateral ventricles (posterior region), or in between (mid-region or centrum semiovale);

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and observed unilaterally or bilaterally (Cornette et al., 2002). They were also the first to include information on the location of the periventricular lesions, which seems particularly important since it is linked to neurodevelopmental outcomes (Guo et al., 2017). However, scans were performed only once, within a range of 1 to 100 days after birth in preterm and at term neonates. The lesions could therefore be underestimated since the scans were taken near term (Martinez-Biarge et al., 2016). Severity of white matter injury in Miller's classification (2003) is based on number, size and hemispheric involvement (Miller et al., 2003). These authors performed MRIs in the first few weeks of life and at TEA but did not include information on the specific location of the lesion in their classification. Commonly used classifications, such as those of Woodward (Woodward et al., 2006a) and Kidokoro (Kidokoro et al., 2013), aimed to include more types of white matter lesions and rank each category independently before calculating a global score. Concerning focal lesions, it should be recognized that both classification rely on a qualitative evaluation based on the neuroradiologist's experience and subjectivity and do not account for the precise location of the lesions. The extent of white matter signal abnormalities is classified as focal (≤ 2 regions per hemisphere) or more substantial (> 2 regions per hemisphere) in the Woodward's classification (Woodward et al., 2006b), and as focal, extensive or linear in the Kidokoro's classification (Kidokoro et al., 2013). These classifications are based on MRI at TEA which is not ideal as severity of the injury may be underestimated with a single MRI at TEA (Martinez-Biarge et al., 2016). More recently, Martinez-Biarge et al. (Martinez-Biarge et al., 2016) have proposed a similar classification, but with serial MRIs (< 2 weeks of age; 2-6 weeks of

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age; at TEA). The extent of white matter signal abnormalities is classified with the number of lesions, with a cut-off of 6 (< or ≥ to 6) lesions.

Overall, the current classification methods examine qualitatively the number, the size and, for some, the location of lesions. The literature also suggests the importance of performing MRI early in life and at TEA to classify PWML in the preterm. Indeed, MRIs at TEA not often disclose the full extent of the lesions and may consequently underestimate their link with the outcomes. If early MRI in preterm babies is generally considered as safe (Merchant et al., 2009; Benavente-Fernández et al., 2010), minor incidents can still arise during and following the procedure such as respiratory instability and hypothermia. Providing an adequate environment together with close monitoring and continuous improvements of MRI guidelines are warranted to avoid such events (Plaisier et al., 2012). As detailed in the next section, it would be of interest to consider the precise localization in addition to injury load for white matter injury assessment to reflect a more accurate outcome This prompts the need for new classification methods. A recent study by Guo et al. (2017) demonstrate the utility of measuring lesion location and lesion load in a quantitative, objective and automatic way, as they were able to associate the volume of white matter injury between different brain lobes and outcomes (Guo et al., 2017).

Insert Table 1

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Table 1. MRI white matter injury classifications in the literature

WM Lesions	Childs et al. (2001)	Cornette et al. (2002)	Miller et al. (2003)	Woodward et al. (2006)	Kidokoro et al. (2013)	Martinez-Biarge (2016)
N, GA	105 preterms (< 37 weeks); 25 at term (≥ 37 weeks)	50 preterms (< 37 weeks); 42 at term (≥ 37 weeks)	32 preterms (< 36 weeks)	167 preterms (< 30 weeks)	97 preterms (< 30 weeks); 22 at term (≥ 37 weeks)	82 preterms (< 35 weeks)
Age at MRI	Between 1 and 42 days of life	Between 2 and 106 days of life	As soon after birth and at discharge or TEA	TEA	TEA (between 36- and 42-weeks GA)	< 2 weeks of age, 2-6 weeks of age and TEA
Specific classification of PWML						
Number	< or ≥ to 3	< 3, 3-10, > 10	< or > to 3			As described in Cornette et al. (2002)
Size	≤ 3mm or > 5mm	< 5mm	< or > 2mm and < or > 5% hemispheric involvement	≤or > 2 regions per hemisphere	Focal or extensive punctate lesions	As described in Cornette et al. (2002)
Location/distribution	PVWM: ≤ or > 2 areas affected or more extensive damage	anterior/ posterior/mid-region of the brain; unilateral/bilateral	PVWM	Whole brain	Whole brain	As described in Cornette et al. (2002)
Appearance		Cluster/linear/mixted-type				As described in Cornette et al. (2002)
Additional variables measured	Cystic lesions	Associated lesions (i.e. oedema, cerebellar/basal ganglia/subarachnoid hemorrhage, cystic PVL, herpetic encephalitis)	Diffuse/cystic lesions	Diffuse/cystic lesions; PVWM volume loss; ventricular dilatation; thinning of corpus callosum; gray matter abnormalities	Diffuse/cystic lesions; WM volume loss; ventricular dilatation; thinning of corpus callosum; delayed myelination	focal/diffuse; cystic/non cystic; WM loss; ventricular dilatation; DWI abnormalities; cerebellar lesions; germinal matric/intraventricular hemorrhage; visual thalamic volume reduction; suggestive changes in gliosis; abnormal myelination of PLIC

Note. DWI, Diffusion Weighted Imaging; GA, Gestational Age; PVL, Periventricular leukomalacia; PVWM, Periventricular White Matter; WM, White Matter

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4. PWML and developmental outcomes

4.1. Outcomes in the preterm infant

The clinical interpretation of outcomes for PWML remains challenging (see Table 2). Indeed, age at birth, at scan, and at outcome vary greatly between studies and within the studies themselves. However, we know that these variables modify significantly the presence, volume and number of PWML (Martinez-Biarge et al., 2016). Most studies detailed the relationship between PWML and outcomes measured at two years of age in infants born prematurely (GA < 37 weeks) or very prematurely (GA < 32 weeks). The MRI was most often performed at TEA, but sometimes as soon after birth as well (Dyet et al., 2006; Kersbergen et al., 2014; Guo et al., 2017).

Adverse motor outcomes, such as cerebral palsy, abnormal muscular tone, low gross and fine motor scores (e.g. grasping, sitting, stacking blocks, and climbing stairs), have been shown to correlate to the number (De Bruïne et al., 2011) and the volume of the PWML (Guo et al., 2017; Tusor et al., 2017). Contrarily, a few studies showed no relationship between motor outcomes and the presence of PWML (Cornette et al., 2002) or the number of PWML (Dyet et al., 2006; Kersbergen et al., 2014). However, the results of these studies should be considered with precaution, as sample sizes were relatively small, thus limiting the confidence of findings (Cornette et al., 2002; Dyet et al., 2006). Moreover, the studies reported small number of PWML, that is less than 9 (Dyet et al., 2006; Kersbergen et al., 2014), while the presence of more than 20 lesions at TEA is considered as a good predictor of poor motor outcomes (Tusor et al., 2017). Finally, lesion volume represents a more objective measure than the number of lesions that can be hard to assess especially in the case of clusters. The volume of the lesion may thus constitute

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a more reliable measure of the lesion load to predict neurodevelopmental outcomes (Guo et al., 2017; Tusor et al., 2017).

Most of the research reported no relationship between language and cognitive abilities and the number (Dyet et al., 2006; Kersbergen et al., 2014) or volume of PWML (Guo et al., 2017; Tusor et al., 2017). There was only one study, to date, that showed cognitive disabilities and behavioral problems in relation to the number of PWML in preterm infants (De Bruïne et al., 2011). However, when considering the location or volume of lesions, it appears that frontal lesions predicted poor language and cognitive outcomes (Guo et al., 2017), while PWML may increase the risk of motor deficits (Rutherford et al., 2010; Tusor et al., 2017) when located in the corticospinal tract (including the posterior limb of the internal capsule), or may induce visual impairments when PWML were located in optic radiations (Rutherford et al., 2010; De Bruïne et al., 2011; Li et al., 2017). Unfortunately, lesion location was not taken into account in most of the studies that examined the relationship between PWML and neurodevelopmental outcomes, despite its importance.

Only one study in the literature looked at outcomes at school age of very preterm infants (≤32 weeks of GA). They assessed the link between isolated PWML at birth or TEA and neurodevelopmental outcome between 9 and 14 years (Arberet et al., 2017). Compared to the youths without lesion at birth or TEA, children with isolated PWML began to walk later and had a higher frequency of cerebral palsy, medical equipment requirements, need for special care and dyspraxia (Arberet et al., 2017).

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4.2. Outcomes in full-term infants

The relationship between PWML and outcome in infants born at term has been scarcely examined, as PWML are more notorious in the preterm infants. In the Cornette's cohort, there were only 2 infants born at term with PWML, and no associations were found with the outcomes (Cornette et al., 2002). In the context of infants with congenital heart disease, Beca et al. (2013) have examined outcome of infants with WMI (described usually as punctate lesions) preoperatively and/or post-surgery, at 2 years of age. New postoperative punctate lesions were associated with mortality after PICU discharge but not with neurodevelopmental outcome at 2 years of age (Beca et al., 2013). That being said, more studies focusing on the effect of PWML in full-term infants and their developmental outcome are warranted.

Insert Table 2

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Table 2: Studies of the link between PWML and outcomes in infants

Study (design)	N	GA (mean, weeks)	Age at scan (postmenstrual weeks)	MRI	Corrected age at outcome and outcome measurement	PWML classification	Exclusion	Results
Cornette et al. (2002)	Preterm: 15; 10 for outcome At term: 2	31/40	35	1.5T (Philips)	29.5 months Clinical evaluation: gross motor, person-social, language, and fine motor-adaptive realms	*Cornette's classification*	Perinatal asphyxia, convulsions, congenital or posthemorrhagic hydrocephalus, dysmorphism, PVL, persistent echodensity, congenital infection, mitochondrial disorder, other cerebral lesions	Neurodevelopmental outcome is favorable: no subject died, no motor impairments, and only a slight delay in language development was seen in one girl at the age of 34.1 months
Dyet et al. (2006)	Very preterm (<30w): 17	27.4	Soon after birth and TEA (36w)	1T	Between 18 and 36 months of age GMDS	Number (between 1 and 9; mean = 2)	Infants with significant focal lesions, HPI, basal ganglia and thalamic lesions, cerebellar hemorrhage, congenital abnormalities	No relationship between the number of lesions and developmental outcome
De Bruine et al. (2011)	Very preterm (<32w): 67 with ≤6 PWML 8 with >6 PWML	29.4	40-44	3T (Philips)	24 months GMFCS; BSID II (MDI; PDI); BSID III; CBCL	Number (≤or>6)	Congenital nervous system abnormalities	The group with six or more PWML, compared to the group with less than 6 PWML, had lower MDI and PDI, more severe motor delay and cerebral palsy, more total behavioral problems and externalizing behavioral problems
Kersbergen et al. (2014)	Preterm: 112	34	GA<28w: ~30w and ~TEA GA>28w: Just after birth and ~TEA	1.5T: Early n=9 TEA n=3 3T (Philips)	15 months (n=100) and 24 months (n=83) GMDS or BSID III	*Cornette's* and *Miller's classifications* Appearance; number (<or≥6)	Congenital abnormalities	Both the relation between initial appearance (i.e., linear or cluster) and between lesions number (i.e. < or ≥ 6 lesions) and neurodevelopmental outcome were not significant
Tusor et al. (2017)	Preterm: 114 with PWML 281 without lesions	30	TEA	3T (14 neonatal units)	20 months GMFCS; BSID III	PWML volumes	other focal cerebral or cerebellar lesions (PVL, IVH > grade 2, porencephalic cyst, hemorrhagic parenchymal infarction	Moderate or severe functional motor disability more common amongst infants with PWML compared to those with no lesions Larger PWML volume correlated with higher GMFCS level and lower BSID III motor scores, but not with BSID III language or cognitive scores

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Guo et al. (2017)	Very preterm (24-32w): 216	27.9	32.1 or TEA (40w)	1.5T (Siemens)	18 months BSID III	PWML volume; location	macrocystic lesions (≥10mm^3), antenatal infections, congenital malformations/syndromes, parenchymal hemorrhagic infarction >2cm	Greater PWML volumes in frontal, parietal, and temporal lobes predicted poor motor outcomes, with injuries in the frontal lobe being the most predictive. Only frontal PWML predicted adverse cognitive outcomes.
Arberet et al. (2017)	Very preterm (<32w): 12 with grade 2-3 54 with grade 1	<32	32-41	1.5T (General Electric)	9-14 years Quality of life (Health Status Classification System Pre School questionnaire)	*Woodward's classification*	genetic disease, congenital malformation, other cerebral lesions	Compared to the youths without lesion at birth or TEA, children with PWML began to walk later and had a higher frequency of cerebral palsy, medical equipment requirements, need for special care and dyspraxia. PWML associated with alterations in mobility item-getting around, dexterity item-using hands and fingers and motor outcomes. No significant difference between the two groups: school attended, frequency of attention and behavioral disorders
Beca et al. (2013)	Infants with congenital heart disease: 57 with PWML; 78 without PWML	No preoperative PWML: 38.9 Preoperative PWML: 38.6	Preoperatively (within ~7 days of life), ~7 days after surgery, ~3 months of age	1.5 or 3.0T Magnetom Avanto scanner	24 months (more or less 6 weeks) BSID III	WMI, usually PWML Number and Size: Mild (≤3 foci and ≤2mm) Moderate (>3 and ≤10 foci or >2mm) Severe (>10 foci) Also classified stroke, CSF and brain maturity	<36w GA, genetic or malformation syndromes known to be associated with abnormal neurodevelopment, if they required extracorporeal membrane oxygenation before surgery	New PWML postsurgery associated with mortality after PICU discharge. No association with neurodevelopmental outcome

Notes: GMDS, Griffiths Mental Development Scales; BSID III, Bayley Scales of Infant and Toddler Development, third edition; MDI, Mental Developmental Index; PDI, Psychomotor Developmental Index; GMFCS, Gross Motor Function Classification Score ; CBCL, Child Behavior Checklist; IVH, Intra-Ventricular hemorrhage; GA, Gestational age

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5. MRI acquisition protocols and anatomo-pathological correlations of PWML

5.1. Quality of MRI sequences need to be defined and standardized

As mentioned previously, PWML are generally identified using conventional MRI sequences (T1-weighted or T2-weighted images). However, sequences vary across studies and may lead to underestimate PWML, particularly regarding the resolution of acquisitions. Considering the small size of PWML, it is crucial to establish a uniform protocol in order to correctly assess them. For instance, the voxel size affects directly the quality of resolution, and it is more difficult to identify PWML on T1-weighted and T2-weighted images with bigger voxels. As shown on Figure 2, PWML should be analyzed on higher resolution 3D T1 imaging with an isotropic resolution of 1 mm^3 or below. (Figure 2).

Insert Figure 2

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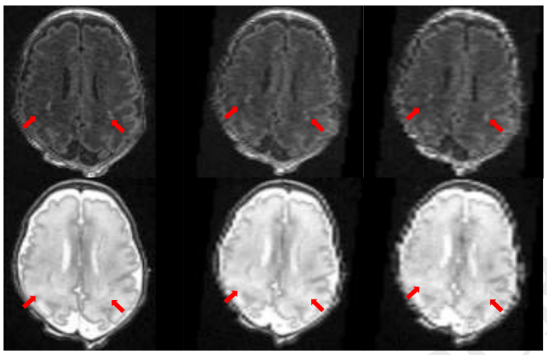

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a:

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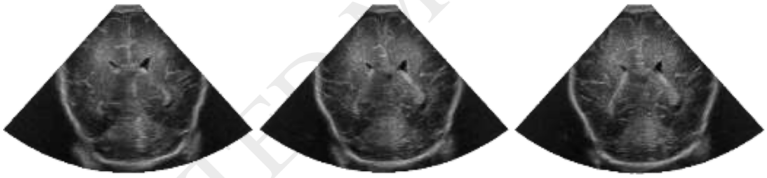
Superior



Inferior

Figure 2a Top: Punctate white matter lesions (red arrows) as seen in 3D T1-weighted (top row) and T2-weighted (bottom row) sagittal acquisitions in a preterm infant born at 29 weeks of gestation and scanned at 33 weeks, compared to the same data simulated across varying degree of down-sampling anisotropic voxel resolution, mimicking typical clinical acquisition resolution. Data were down-sampled using SPM12 with 7th degree B-spline interpretation. Note how PWML become much less distinguishable with the reduction in resolution on T2-weighted imaging and harder to determine with certitude on T1-weighted imaging.

Figure 2b Bottom: Same patient as seen in three consecutively acquired coronal ultrasound image three days before the MRI scan. Location of the coronal slice is approximate with the PWML location in MRI. Note that clinical review of the ultrasound image did not notice any irregularity.

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Aside from conventional MRI sequences, it might be of great importance to examine microstructural MRI parameters to quantify white matter microstructure (Groeschel et al., 2016), as diffusion weighted imaging (DWI), susceptibility-weighted imaging (SWI) and MR spectroscopy (MRS) and thus may provide complementary information to characterize start identifying subsets of PWML (Benders et al., 2014; Groeschel et al., 2016).

5.2. Multimodal imaging to identify subsets of PWML

PWML with a linear appearance, compared to PWML with a cluster appearance, seem to have hemorrhagic origin (Kersbergen et al., 2014). On conventional MRI, PWML with a linear appearance were seen more clearly on early T2-weighted imaging and may correspond to hemoglobin degradation products (Kersbergen et al., 2014). However, their intensity is quite similar to myelinated white matter and thus may be regions with high lipid content. Signal intensity changes on T1-weighted and T2-weighted may also suggest gliotic scarring (Childs et al., 2001; Cornette et al., 2002; Sie et al., 2005; Dyet et al., 2006; Ramenghi et al., 2007; Niwa et al., 2011). Based on DWI sequences, a decreased apparent diffusion coefficient (ADC) may suggest an ischemic origin (Benders et al., 2014) or from a signal distortion due to hemorrhage (Benders et al., 2014). However, it remains difficult to make a distinction based only on conventional MRI or even DWI since signal distortion does not clearly distinguish hemorrhages (Dyet et al., 2006; Niwa et al., 2011; Benders et al., 2014; Kersbergen et al., 2014).

With the complementary use of SWI sequences, it appears possible to distinguish hemorrhagic and non-hemorrhagic types of PWML. Niwa et al. (2011) studied SWI

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signal loss in areas of PWML using conventional MRI. The authors reported a higher percentage of SWI signal loss in preterm infants with intraventricular hemorrhage as a primary diagnosis, compared to those with a primary diagnosis of white matter damage. Most of the PWML in neonates with intraventricular hemorrhage were located near the medullary veins. These areas would be suggestive of petechial hemorrhages compared to PWML in infants with a primary diagnosis of white matter damage for which early gliosis should be considered instead (Niwa et al., 2011). In their single postmortem histopathological analysis, the authors found that local areas with signal loss on T1-weighted and SWI corresponded to hemorrhages (Figure 3A-D) whereas those with an altered T1-weighted signal and normal SWI showed signs of early gliosis without hemorrhagic manifestations (Niwa et al., 2011). Moreover, complementary use of DWI and SWI sequences have shown that cluster-type PWML identified on DWI scans with normal signal intensity on SWI have a restricted mean diffusivity (Rutherford et al., 2010; Kersbergen et al., 2014), suggesting that these areas may also represent infarction occurring from inflammatory or ischemic processes (Cornette et al., 2002; Rutherford et al., 2010; Kersbergen et al., 2014; Wagenaar et al., 2017).

Insert Figure 3

The Brain's Kryptonite: Overview of Punctate White Matter Lesions in Neonates ACCEPTED MANUSCRIPT

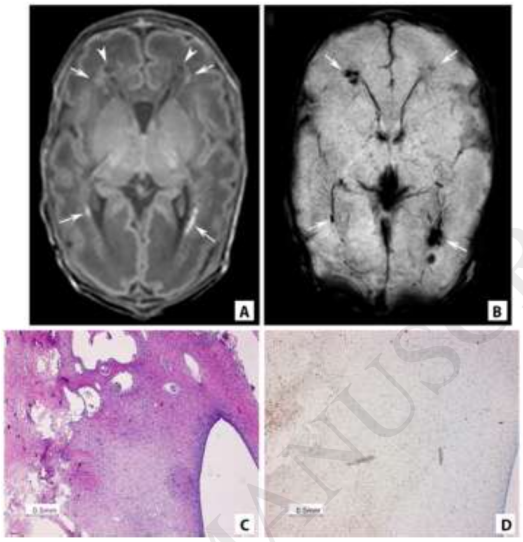

Figure 3. Examination of a preterm infant born at gestational age of 31 weeks. Infant was scanned on day 11 and died at 13 days of age. T1-weighted scan with focal hyperintensities (*arrows*) and cystic regions (*arrowheads*) (A). SWI scan at the corresponding slice with signal loss suggesting the presence of hemorrhage (*arrows*). Note that in this case, these areas closely match those with signal abnormalities as seen on T1-weighted scan. Post-mortem histology shows cystic and hemorrhagic areas in the white matter (C) as well as activated microglia cells and macrophages surrounding those areas by using CD68 staining (D). Signs of early gliosis are also revealed with positive GFAP staining observed in the periventricular area in regions corresponding to abnormalities on T1-weighted imaging and normal SWI (not available). Adapted from (Niwa et al., 2011).

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5.3. Microstructural alteration in the context of PWML

Diffusion tensor imaging allows the study of anisotropy (e.g. Fractional Anisotropy, FA) and diffusivity (radial, axial) for each voxel, and to create images reflecting various properties of diffusion (Tournier et al., 2011). Increased radial diffusivity has been correlated with myelin loss, whereas decreased axial diffusivity has been linked to axonal injury (Lodygensky et al., 2010, 2014; Tournier et al., 2011).

Using tract-based spatial statistics and probabilistic tractography, FA values at TEA were lower in the posterior limb of the internal capsule, cerebral peduncles, decussation of superior cerebellar peduncles, and pontine crossing tract in preterm infants with PWML, compared to preterm infants without lesion (Bassi et al., 2011; Tusor et al., 2017). Moreover, FA at TEA in corticospinal tracts was negatively correlated with the number of PWML (Bassi et al., 2011), and FA at TEA in the corona radiata and posterior limb of the internal capsule were negatively correlated with PWML volume (Tusor et al., 2017). Radial diffusivity (RD) values at TEA were higher in the centrum semi-ovale, corona radiata, posterior limb of the internal capsule, and arcuate fasciculi in infants with PWML, when compared to preterm infants without lesion suggesting impaired myelination (Tusor et al., 2017). Moreover, RD at TEA in the corona radiata and posterior limb of the internal capsule were positively correlated to PWML volume (Tusor et al., 2017). Li et al. (2017) reported reduced FA, increased RD in the centrum semi-ovale and/or corona radiata, white matter near the trigone of the lateral ventricles, splenium of the corpus callosum, and optic radiation (Li et al., 2017). However, these results may be considered with caution as white matter changes were reported in infants

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with grade III PWML only, and not in infants with grade I or II PWML (Li et al., 2017). Areas of reduced FA in the corticospinal tracts with PWML were also more extensive than the lesions visible on conventional MRI (Bassi et al., 2011). Overall, FA and RD changes reflect an altered brain microstructure with axonal injury and diffuse damage with myelin loss indicating that PWML lead to broader damage on the brain's microarchitecture compared to what only conventional MRI would suggest. Hence, PWML may be the epiphenomenon of a wider underlying injury and dysmaturation process (Bassi et al., 2011; Benders et al., 2014).

5.4. Modified hemodynamics and metabolite concentrations

Using advanced MRI techniques such as Phase-Contrast MR angiography and T2-Relaxation-Under-Spin-Tagging, it is possible to extract information on cerebral hemodynamics of neonates (Lu and Ge, 2008; Van Kooij et al., 2010; Benders et al., 2011; Varela et al., 2012; Xu et al., 2012; Jain et al., 2014; Liu et al., 2014). In a recent study, valuable physiological biomarkers of brain functioning such as the value of cerebral metabolic rate of oxygen (CMRO2), global cerebral blood flow (CBF), oxygen saturation fractions in venous blood and oxygen extraction fraction were quantified in infants with PWML (Qi et al., 2018). When compared to infants without PWML, those with PWML had lower oxygen consumption as seen with a higher oxygen saturation fractions in venous blood and a lower oxygen extraction fraction, independently of age and maturation (Qi et al., 2018).

Using magnetic resonance spectroscopy (MRS), Wisnowski and colleagues examined glutamate and glutamine concentrations in the parietal white matter, in infants

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with PWML (Wisnowski et al., 2013). They observed that glutamine concentration was significantly increased in association with PWML, compared to those without PWML, reflecting perturbation of glutamate-glutamine homeostasis (Wisnowski et al., 2013). N-acetyl aspartate concentration, a biomarker commonly used to estimate neuronal integrity (Patel and Clark, 1979; Urenjak et al., 1992; Clark, 1998; Wisnowski et al., 2013), was decreased on MRS in infants with PWML, compared to those without PWML (Wisnowski et al., 2013) reflecting poor neuronal maturation (Sánchez-Gómez et al., 2003; Volpe et al., 2011; Wisnowski et al., 2013).

5.5. Inflammation and myelin disruption described in histology cases

Regions of PWML occur in unmyelinated white matter (Niwa et al., 2011). In the case of non-hemorrhagic PWML specifically, this disturbance may result from inflammatory or ischemic processes (Kersbergen et al., 2014). This may be due to a defect in cerebrovascular autoregulation leading to cerebral ischemia (Volpe, 2001; Back et al., 2005) or to neuroinflammation following pre and/or postnatal infection (Dammann et al., 2001; Hagberg et al., 2002; Back et al., 2005). Findings in the context of congenital heart disease also suggest a principal ischemic origin since PWML were common in infants going through surgery without cardiopulmonary bypass. These subtle white matter lesions may also be related to gliosis, necrosis, mineralization, oligodendroglia damage or axonal damage (Van De Bor et al., 1987; Dyet et al., 2006; Niwa et al., 2011; Liu et al., 2014; Cizmeci et al., 2018).

In a rare case-study, the histological examination of post-mortem tissues in an infant with PWML revealed microglial infiltration in areas corresponding to PWML (Rutherford et al., 2010) (Figure 4). Such a result is well in line with the hypothesis

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postulating that neuroinflammation may significantly contribute to the occurrence of these lesions.

Insert Figure 4

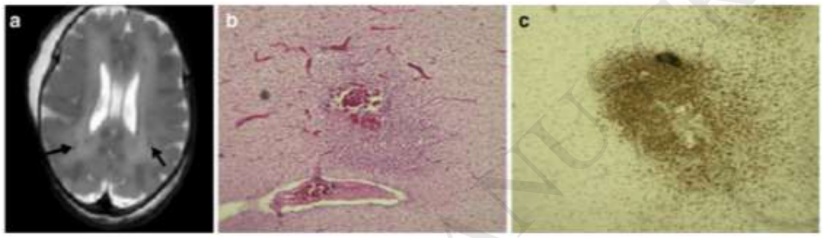

Figure 4: Post-mortem images of a preterm infant born at 34+6 weeks gestation, who died at 9 days of age. Hypotense focal lesions are observed at the T2-weighted MRI image and are mostly localized posteriorly (*arrows*) (a). These regions constitute perivascular lesions (b) which correspond to activated microglia aggregation (CD68 positive cells) (c). Adapted from (Rutherford et al., 2010).

To better understand the neuropathological substrate there are a few available animal models of PWML. Using a fetal lamb model replicating inflammatory injuries in the preterm brain by lipopolysaccharide exposure, patterns of diffuse and focal PWML lesions were detected by ex-vivo MRI (Dean et al., 2011; Van De Looij et al., 2012). Histopathological assessment revealed that these PWML corresponded to activated microglial cells as shown in Figure 4 (Dean et al., 2011). Fetal lamb exposure to lipopolysaccharide caused PWML characterized by cell necrosis with acide fuchsin/thionin staining, These lesions showed loss of astrocytes and neurofilaments, neuronal amyloid precursor protein (APP) accumulation, and a dense accumulation of

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activated microglia (Dean et al., 2011; Van De Looij et al., 2012) (Figure 5). In a distinct fetal lamb study exposed to global ischemia, PWML were also found to be composed of microglia accumulation using Iba1 staining and a decreased astroglial staining (Riddle et al., 2011) (Figure 6). Both studies mimick human autopsy data (Rutherford et al., 2010; Niwa et al., 2011). Van de Loijj et al. (2012) suggested that T2 hypointensities and T1 hyperintensities in focal lesions from the preterm ovine model could derived from high-lipid content in the activated microglia or the high cell density in the area (Van De Looij et al., 2012).

Insert Figures 5 and 6

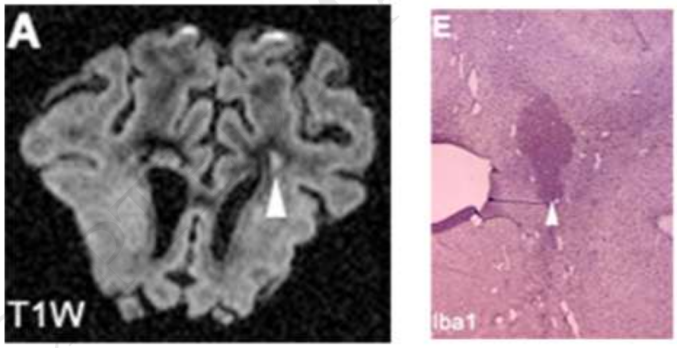

Figure 5. Ex-vivo coronal images of a lipopolysaccharide-exposed ovine fetus. A: An hypertense T1-weighted MRI image exposes a focal periventricular white matter lesion (arrowheads). This region shows a dense accumulation of microglia/macrophage as revealed by Iba1 staining (E). Adapted from (Dean et al., 2011).

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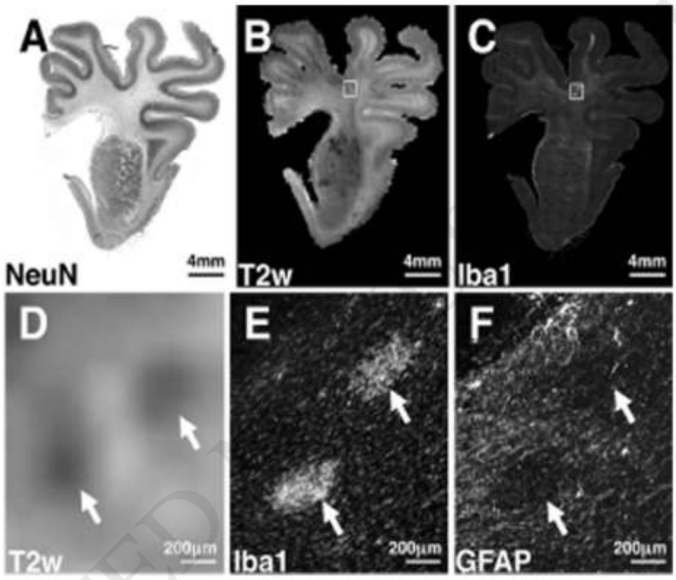

Figure 6. Brain images from a preterm fetal ovine model. Aligned MRI and histological sections allowed the detection of focal lesions on a 2-week ischemia survivor. A: NeuN (neuronal nuclei) staining without apparent lesion; B and C: T2-weighted image in B shows apparent white matter signal abnormalities (inset box) corresponding to a positive Iba1 staining in the aligned image of Figure C. The inset boxes presented at higher magnification in figures D (T2-weighted image), and Figures E and F (immunostaining), respectively, show two abnormal white matter hypointensities in Figure D, which correspond with an intense microglial/macrophage infiltration (Iba1 positive cells, arrows) in Figure E, but reduced astroglial staining at the center of the lesions (GFAP positive cells, arrows) in Figure F. Adapted from (Riddle et al., 2011).

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7. Conclusion

PWML in preterm infants were found in some studies to be associated with long-term neurodevelopmental deficits. Additional studies are warranted to increase knowledge about the etiology and the impact of these lesions on the motor and cognitive development. PWML are also associated with widespread microarchitecture modifications of the white matter and could be a marker of a broader underlying lesion. Future clinical studies should include an MRI as early as possible in the child's life, with larger cohorts and sequential high-resolution multimodal imaging. Indeed, this will allow a better assessment of the true incidence, the distinction of the different PWML subtypes and will improve our understanding of the specific long-term impact. It is very likely that PWML will establish itself as an important biomarker and a target for future neuroprotective trials.

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Potential Conflicts of Interest

None declared

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Authors' contributions

AN was involved in carrying out primary research, drafting and revising critically the manuscript. YD prepared the neonatal MRI data to provide the illustration of Figure 1 and 2. SS, IL, YD, DL, and GL were involved in drafting the manuscript and revising it critically. GL participated in the design and coordination of the paper. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

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